The Thesis Committee for Ilane Ximenes Frota Máximo
Certifies that this is the approved version of the following thesis:

The impact of nonsteroidal anti-inflammatory drugs on endocrine therapy outcomes in breast cancer patients

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Linda A. deGraffenried

Stephen D. Hursting

The impact of nonsteroidal anti-inflammatory drugs on endocrine therapy outcomes in breast cancer patients

by

Ilane Ximenes Frota Máximo, B.Sc.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Arts

The University of Texas at Austin

May 2013

Dedication

I dedicate this thesis to my beloved parents and husband. Thank you for your love, for always being by my side and believing in me.

I would also like to dedicate this work to the patients struggling with breast cancer.

Acknowledgements

First and foremost, I would like to thank God for the gift of life and the blessings He has given me. Thank you for helping me throughout my graduate school years, for my family support and for the friends you have given me in Austin.

I would like to thank my primary supervisor, Dr. Linda deGraffenried, for welcoming me into her laboratory and offering me such a meaningful project to work. Thank you for being a caring advisor, for your words of wisdom, guidance and support. I admire you as a woman and professional.

Thank you to my supporting supervisors, Drs. Hursting and Brenner. Dr. Stephen Hursting, thank you very much for your support and guidance during my graduate school years. Thank you, Dr. Andrew Brenner, for being an exemplary medical oncologist, for answering my questions and feedback.

I am very grateful to Dr. Murali Beeram for his insights and for being a role model professional. I would also like to extend my thank you to the staff and doctors at the clinics in San Antonio for helping me pursue my study and for their kindness.

Thank you to the great professors and lectures at UT at Austin that enhanced my nutrition knowledge, especially Jeanne Freeland-Graves, Monica Meadows, Lydia Steinman, Charlotte Herzele, Michele Forman, Jaimie Davis, Ladia Hernandez, Monica Milonovich and Katherine Southworth.

I would like to thank my nutrition peers for their support, words and hugs, especially Renee Stubbins, Shruti Apte, Laura Bowers, Dave Cavazos, Cindy Chen, Alejandra DeAngulo, Gloria Galvan, Ramona Price, Yeyi Zhu and Lauren Mangini.

I am very grateful for the help of the statisticians Sally Amen, Erica Hale and Michael Mahometa.

I would also like to express my gratitude to the assistance given to me by Kathy McWilliams, Miriam Pashby and Barbara Satterwhite during my graduate school.

I would like to acknowledge my Austin friends, who never failed to motivate and encourage me to do what I love. A special thank you very much to Emanuel and Vanessa Carneiro; Ivy and Darlan Girão; Renata and Ricardo Conceição; Debbie, Harry and Virginia Roberts; Ignácio Gallardo; Reza and the Koohrangs; Charlotte and Eduardo Garza; Jessa and Rodolfo Barniol; Haotian Wu; Maria Eva and Patrícia Campos.

A special thank you to my family and my husband's family (grandparents, aunts, uncles, cousins, nephews), especially Ivna, Joaquim, Amanda, Raul, Heloísa and Vicente. Each one of you has a special place in my heart and I am sure you know it. Thank you for everything.

I have no words to express my deepest gratitude to my parents. Thank you so much for the education you have given me, for helping me pursue my dreams, for the encouragements and unconditional love. You are my heroes and my true gifts from God. I am blessed and proud to be your daughter. And thank you for giving me a sister, who always also motivated me through this journey.

Finally, thank you to my husband, my love, companion and best friend. Thank you so much for being by my side through the happy and difficult times, for always holding my hand, for being such a role model in my life. I love and admire you with all my heart.

Abstract

The impact of nonsteroidal anti-inflammatory drugs on endocrine therapy outcomes in breast cancer patients

Ilane Ximenes Frota Máximo, M.A.

The University of Texas at Austin, 2013

Supervisor: Linda A. deGraffenried

Obesity is a known risk factor for postmenopausal breast cancer, and is associated with worse disease prognosis in pre- and postmenopausal women. Adjuvant hormonal therapies improve disease prognosis in obese women, but many still recur. Given that obesity induces inflammation and increases levels of cyclooxygenase-2 (COX-2) enzyme, resulting in tumor proliferation, this retrospective study investigated if women on anti-inflammatory drugs would have improved disease outcomes by reduced production of prostaglandins by COX-2 pathway. Four hundred and forty women treated for invasive breast cancer in San Antonio clinics were included. Cases were classified as

NSAID users if notes included daily use of aspirin, ibuprofen, celecoxib or another COX-2 inhibitor; patients were categorized as NSAID nonusers if they were not taking any NSAIDs, or if they used COX-2 drugs for pain as needed rather than daily. Patients on NSAIDs were more likely to be older, be slightly more obese and postmenopausal. NSAID and NSAID nonusers did not statistically significantly differ in regards to BMI categories, tumor stage, hormone receptor status, type of invasive tumor, ethnicity/race and type of surgery. NSAID users had significantly less recurrence rates compared to nonusers ($p=0.05$). Further, time to disease progression was delayed by almost 28 months in patients who were NSAIDs users. Although this trend was non-significant statistically due to low number of total recurrences, it is promising in the clinical setting. In a logistic regression model using NSAID use, BMI categories and hormonal therapy drug as independent variables to predict recurrence, use of NSAID was only statistically significant in the univariate model. Overweight women were more likely to develop recurrence than normal weight when holding NSAID use and endocrine therapy constant. Obese women had increase recurrence risk, but the trend was not statistically significant. Females using aromatase inhibitors were less likely to recur than those on tamoxifen. The results of this exploratory study had limited power to determine multiple modulating factors, but because they suggest a major clinical benefit, further analyses in a larger sample size are needed to confirm these findings.

Table of Contents

List of Tables

List of Illustrations

Chapter 1: Introduction

Obesity is a significant public health problem not only in the United States but also worldwide. Over 35% of Americans are obese (1), and this percentage is alarming because obesity increases the risk of various cancers, including postmenopausal breast cancer (2). Obesity is also associated with worse disease prognosis in pre- and postmenopausal breast cancer patients (3, 4).

Obesity is considered an inflamed state because of the secretion of adipokines and cytokines by the adipose tissue. These pro-inflammatory agents seem to be indirectly associated with decreased survival in obese breast cancer patients (5). While adjuvant hormonal therapy has helped increase the number of breast cancer survivors, many are still at risk of breast cancer recurrence, especially obese females (6, 7). Therapies to improve breast cancer patient outcome in conjunction with hormonal therapy need to be further explored.

Daily use of nonsteroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase-2 (COX-2) inhibitors, such as aspirin, celecoxib and ibuprofen, have been associated with reduced risk of cancers, including breast cancer (8, 9). However, the data is limited and inconclusive regarding NSAIDs use and disease outcomes in breast cancer patients. This study aimed to enhance knowledge in breast cancer outcomes by analyzing whether the use of NSAIDs would decrease breast cancer recurrence rates in women treated with adjuvant hormonal therapy.

The main hypothesis of this thesis was that breast cancer patients who use COX-2 inhibitors on a daily basis have improved disease prognosis due to inhibition of COX-2-derived prostaglandin E_2 (PGE_2) production.

Chapter 2: Literature Review

OBESITY AND BREAST CANCER

Obesity has been a major public health issue in the United States for over 30 years. Currently, more than one third of American adults are classified as obese (1), which is defined as body mass index (BMI) of or higher than 30kg/m^2 (10). Obesity is a known risk of postmenopausal breast cancer (2, 11-13), and it is also related to worse disease outcomes in pre- and postmenopausal females (4, 5, 14-17).

Findings of the Endogenous Hormones and Breast Cancer Collaborative Group showed that patients with BMIs between 27.5-29.9 kg/m^2 had a 1.62-fold risk of breast cancer risk, and those with BMI $\geq$ 30 kg/m^2 had a 1.36-fold increased risk when compared to women whose BMI were below 22.5 kg/m^2, respectively (p=0.004) (2).

The Million Women Study found that postmenopausal women had increased risk of breast cancer as BMI increased. Overweight and obese women had 1.21-fold (95% confidence interval [CI], 1.13-1.29) and 1.29-fold increase (95% CI, 1.22-1.36) in breast cancer incidence, respectively, compared to normal weight women (18).

A large cohort with over 900,000 adults reported that risk of breast cancer mortality for women increased with each consecutive increase in BMI units. Females classified with obesity levels I, II and III had risk of death increased by 1.63, 1.70 and 2.12 times, respectively, when compared to females of normal weight (3).

Similar evidence was observed in a study with more than 420,000 postmenopausal females, which found that the risk of breast cancer death increased with increase in BMI, and that being overweight (BMI between 25.0 and 29.9kg/m^2) and obese increased the rate ratio (RR) of breast cancer mortality by 1.33 and 1.60, respectively, in comparison to females of normal body weight (< 25kg/m^2) ($p < 0.0001$) (17).

In regards to disease prognosis, a French cohort study with 14,709 breast cancer cases reported that obese women had worse disease outcome for metastasis recurrence in both univariate (hazard ratio [HR], 1.32; 95% CI, 1.19-1.48) and multivariate survival analysis (HR, 1.12; 95% CI, 1.00-1.26) compared to non-obese women (BMI was classified as below 30kg/m^2) (12).

Moreover, a prospective study that followed 923 women for 10 years after disease diagnosis found that 32% of non-obese women were at risk of recurrence compared to 42% of obese women. Further, when only cases without lymph node metastases were examined, 19% non-obese patients developed recurrence compared to 32% of obese patients (19). Finally, a recent meta-analysis concluded that obese women had worse disease survival (HR, 1.33; 95%CI 1.21-1.47) than non-obese women (14).

One of the mechanisms by which obesity is thought to promote a more aggressive disease in breast cancer patients is by the higher levels of local estrogen. Approximately 60 to 75% of breast tumors are estrogen receptor (ER) positive (20-22), and obese postmenopausal women are more likely to develop ER-positive breast cancer (23, 24), suggesting their disease is driven by estrogen. After menopause, the adipose tissue is the main source of estrogen, which is produced by the conversion of androgens to estrogens by the enzyme aromatase (25).

Two types of endocrine therapies are given to patients who have ER-positive breast tumors: tamoxifen and aromatase inhibitors (26, 27). Hormone-dependent breast cancers are typically associated with better clinical response to anti-estrogen therapies, but still 13% of stage III breast cancer patients are at risk of recurrence (28).

Tamoxifen is the first line of endocrine therapy for premenopausal women, and was the only drug available to treat breast cancer between 1973 and 2000 (26). This drug is an example of a selective estrogen receptor modulator (SERM) because it works by

blocking estrogen from binding to its receptor in the breast tumor. Since estrogen acts as an agonist for the ER in the bone and uterine tissue, but as an antagonist in the breast, this drug prevents osteoporosis in women, but can increase risk of other gynecological cancers (29).

Aromatase inhibitors are currently the most commonly used hormonal therapy among postmenopausal breast cancer patients (30, 31). These drugs suppress aromatase activity, stopping the conversion of androgens to estrogens. The main prescribed drugs are Anastrozole (Arimidex®), Exemestane (Aromasin®) and Letrozole (Femara®). Patients using aromatase inhibitors are prone to osteopenia and osteoporosis because the reduced levels of estrogen in the body reduce bone density (27).

Few studies have analyzed the impact of BMI categories on endocrine therapy outcomes. Researchers of the Arimidex, Tamoxifen Alone or in Combination (ATAC) trial analyzed the influence of BMI on hormonal therapy efficacy. The analysis with 5,172 postmenopausal breast cancer cases, who were estrogen receptor (ER) positive, concluded that females with a BMI above 35 kg/m^2 had higher breast cancer recurrence rates compared to females with a BMI below 23 kg/m^2 (HR, 1.39; 95% CI, 1.06-1.82) after treatment with hormonal therapies (7).

Additionally, analysis of the BRENDA-cohort, a German retrospective study, showed that obese patients did worse on aromatase inhibitors in comparison to tamoxifen (32). Recent analysis with 6885 patients from three clinical trials also observed that obese women who were ER-positive and treated with endocrine therapy (aromatase inhibitors) and chemotherapy had poor disease outcomes. This trend is not seen in patients with other tumor cancer subtypes (6).

Multiple cancer-promoting pathways are activated in association with the obese state, including the Insulin-like Growth Factor-1 (IGF-1) and leptin pathways (33). Activation of the IGF-1, leptin and other growth factor pathways induce proliferation as well as survival signaling, most likely contributing to the lower response rates observed in the obese patient population to both endocrine as well as chemotherapy. However, recent data from our laboratory (unpublished) as well as from the Dannenberg group suggest that obese postmenopausal women may also fail treatment because of the inflammation caused by their obese state.

OBESITY-INDUCED INFLAMMATION

Adipose tissue is metabolically active and secretes adipokines, cytokines and hormone-like agents that contribute to inflammation (34). Studies have found that obese women produce more COX-2 enzyme than non-obese (35), which has been correlated with worse disease prognosis (36-39). PGE_2 formed by COX-2 from arachidonic acid, increases aromatase activity (37), resulting in enhanced estrogen receptor activity, and, ultimately affecting tumor development (40) and growth. (41).

Work by Dannenberg's group showed that expression and activity of the enzyme aromatase were correlated with increased levels of COX-2 and PGE_2 levels in the breast tissue of overweight and obese females at high risk of developing or who had breast cancer (35, 42). Preclinically, the authors found that release of fatty acids from adipocytes stimulated the synthesis of the inflammatory agent COX-2 by macrophages, which in turns leaded to PGE_2 formation that upregulates activity of aromatase, estrogen receptor and impacts tumor growth (43).

Because COX-2 inhibitors suppress production of PGE_2, it would be expected that use of NSAIDs would improve disease outcome in obese patients treated with aromatase inhibitors by decreasing inflammation and estrogen production that affect cell proliferation (Illustration 1).

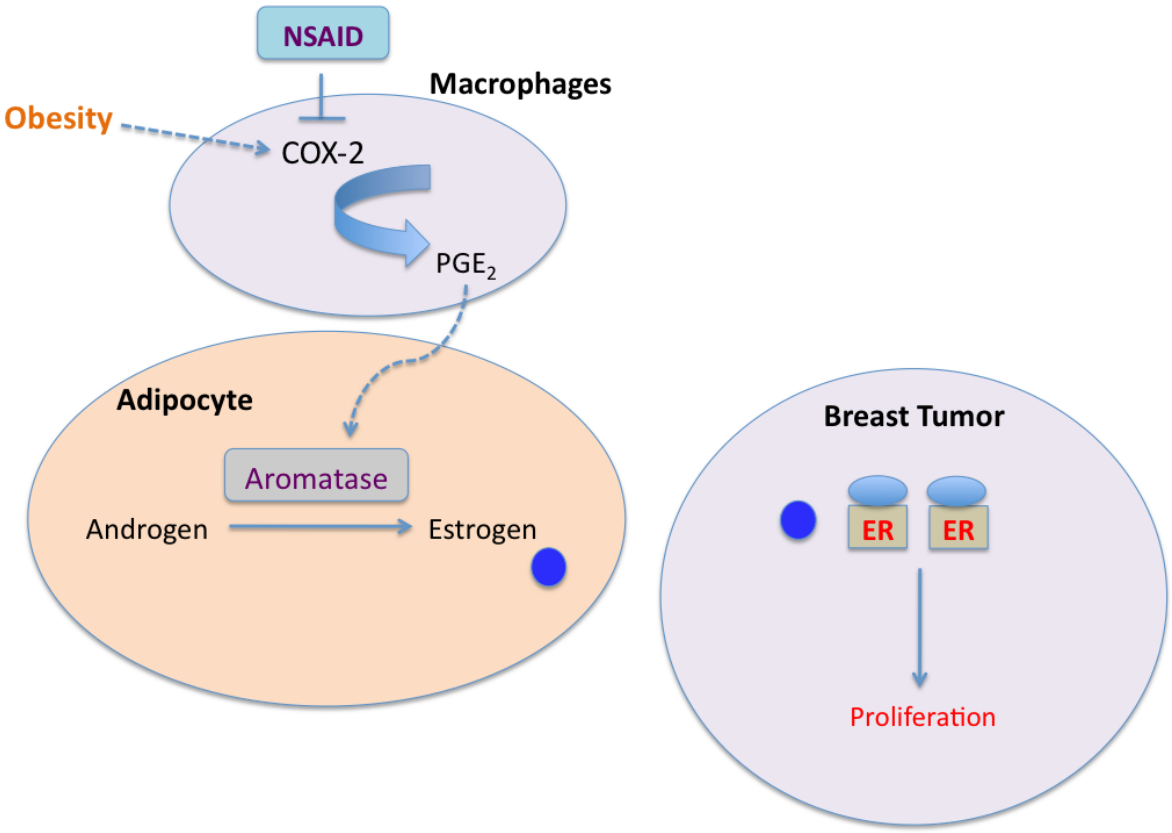

Illustration 1: Proposed model of how the use of NSAIDs would be related to improved disease outcomes. Obesity increases COX-2 activity, which in turn produces PGE_2 from arachidonic acids. PGE_2 stimulates aromatase activity via cAMP pathway. Increased aromatase activity will produce more estrogen from androgens in the fat adipose cells. Higher estrogen synthesis in the adipocyte will also increase binding of estrogen in its receptor sites, thus leading to more tumor proliferation.

ANTI-INFLAMMATORY DRUGS AND BREAST CANCER

The use of anti-inflammatory drugs to prevent cancer incidence is most well-known in research with colorectal cancer (44-46). Numerous studies have investigated the association between NSAIDs and breast cancer incidence. A recent meta-analysis including 38 breast cancer studies concluded that the use of NSAIDs was associated with lower breast cancer risk (RR, 0.88; 95% CI, 0.64-0.97). Aspirin intake was also related to a decreased risk of the disease (RR, 0.87; 95% CI, 0.82-0.92) (9). However, the association remains inconclusive when breast cancer recurrence and/or survival are investigated.

In the Life After Cancer Epidemiology (LACE) cohort conducted with 2,292 survivors of breast cancer, the authors reported a negative and statistically significant association between breast cancer recurrence and regular ibuprofen use (RR, 0.56; 95% CI 0.32-0.98), which was defined as intake at least three days per week (47). Although the same association was not seen for aspirin alone (RR, 1.09; 95% CI, 0.74 to 1.61), when ibuprofen was taken combined with other non-aspirin NSAIDs, the risk of recurrence was reduced (RR, 0.56; 95% CI 0.33-0.95), but BMI did not account for differences.

In a Nurse's Health Study analysis, Holmes and colleagues investigated breast cancer recurrence in 4,164 cases diagnosed between 1976 and 2002. The authors concluded that current and frequent use of aspirin (6 to 7 days per week) was related to a statistically significant lower risk of breast cancer mortality (RR, 0.36; 95% CI, 0.24-0.54). A decreased risk of distant recurrence (RR, 0.57; 95% CI, 0.39-0.82) compared to no aspirin use was also observed (48).

The findings from the Iowa Women's Health Study with 591 postmenopausal females indicated that any use of NSAIDs was associated with a lower risk of all causes

of mortality (HR, 0.57; 95% CI, 0.40-0.81). This trend was not observed for breast cancer death after adjustment for confounders (HR, 0.64; 95% CI, 0.39 to 1.05) (49).

Li and collaborators reported that lifetime (categorized as more than 10 days per month) and current use of aspirin (categorized as more than 14 days per month) were not related to breast cancer survival in 1,024 women diagnosed between 1996 and 2001 (50). Regular recent use of ibuprofen decreased death of all causes, but the result was borderline significant after the model was adjusted for confounders (HR, 0.71; 95% CI, 0.50-1.00). However, the use of NSAID was assessed only prior to breast cancer diagnosis, not after diagnosis.

To our knowledge, no studies have analyzed the impact of NSAIDs on disease outcomes among overweight and obese patients treated with adjuvant hormonal therapy.

CONCLUSION

There is enough evidence to support that the use of anti-inflammatory drugs decreases breast cancer incidence. However, the data is inconsistent in regards to use of NSAIDs and breast cancer recurrence. This thesis aimed to investigate if the use of NSAIDs by breast cancer patients treated with endocrine therapy was associated with improved disease outcome. A secondary objective was to examine the influence of NSAID use and obesity in prediction of recurrence.

Chapter 3: Methods

SUBJECTS

This retrospective study included breast cancer female patients diagnosed with invasive breast cancer and whose records were available at the Cancer Therapy and Research Center at The University of Texas Health Science Center San Antonio. Patients' charts from the clinics dated between 01/01/1987 and 31/12/2011 were reviewed to examine the association between NSAIDs use and breast cancer recurrence in women who were estrogen receptor (ER) positive and received hormonal therapy.

Exclusion criteria included women with carcinoma *in situ*; who declined hormonal therapy or were non-compliant with drug treatment; patients whose therapies were unavailable from charts; patients who stopped using adjuvant therapy due to insurance issues or health problems; triple negative breast cancer cases; patients who had breast cancer metastasis at the time of diagnosis; patients whose dates of breast cancer diagnosis were not available.

DESIGN

This was an exploratory study that used retrospective data from patient charts to investigate if women classified as users of NSAIDs had better disease outcomes compared to nonusers when treated with adjuvant endocrine therapies. Informed consent was obtained from the patients. The study was approved by the Institutional Review Board.

DATA EXTRACTION AND CLASSIFICATION

Information regarding demographics, tumor stage, estrogen and progesterone receptors, and medications were extracted from the patients charts. Weight at baseline was recorded, but when unavailable, the earliest weight that appeared on the charts was used. Premenopausal and perimenopausal women were grouped and classified as premenopausal because they receive tamoxifen as their first-line of hormonal therapy (26, 51, 52).

Women were classified as users of NSAIDs if follow-up or progress notes include *daily* use of aspirin, ibuprofen, celecoxib, naproxen, meloxican or another COX-2 inhibitor in the list of medications. Patients whose medication list stated use of analgesics that do not inhibit production of PGE_2 (53), such as acetaminophen and hydrocodone, as well as any COX-2 inhibitor used p.r.n. (as needed for pain) were considered NSAID nonusers.

Recurrence was defined as any local, contralateral, distant tumor or metastasis of the primary breast cancer. Second primaries were not classified as recurrent breast cancer.

STATISTICAL ANALYSIS

The data was examined for normality. Duplicates were also analyzed to guarantee that one patient was not recorded twice in the event of referrals or having transferred medical care to the other clinic. Descriptive statistics (frequencies, means and standard deviations) were used to describe the data. Pearson's chi-squared tests were used to analyze categorical variables (e.g., recurrence yes/no, postmenopausal vs. premenopausal status) of daily NSAID and NSAID nonusers. Student's t-test were used to examine mean differences in numerical variables (e.g. BMI, time to recurrence). Wilcox non-parametric

test was used to examine if time to recurrence differed between NSAID users and nonusers that developed disease recurrence (44 patients). *A priori* confounding factors included were age at diagnosis and tumor stage. Logistic regression using odds ratios (OR) were used to predict recurrence (dichotomous yes/no). Significance level was set at p-value < 0.05, p-values were 2-sided and 95% CI. Statistical analyses were performed in R Foundation for Statistical Computing (version 2.10.1, Vienna, Austria, 2009).

Chapter 4: Results and Discussion

RESULTS

Patient Characteristics

This retrospective study consisted of 440 women diagnosed with invasive breast cancer who qualified to enter the study. Women whose hormonal therapy treatment and medications could not be obtained from the charts, those who did not seek treatment until the disease was already metastatic, who refused adjuvant therapy or were non-compliant with treatment were not included. Because underweight women might have higher risk of recurrence and/or decreased survival compared to those of normal weight (54-56), three underweight women were excluded from the analysis, not combined with normal weight patient group.

Patients on NSAIDs were more likely to be older and postmenopausal (**Table 1**). NSAID and NSAID nonusers did not statistically significantly differ in regards to BMI categories, tumor stage, hormone receptor status, type of invasive tumor, ethnicity/race and type of surgery.

NSAID users had lower recurrence rates compared to nonusers ($p=0.05$), with recurrence rates being reduced by half in NSAID users. Further, patients on NSAIDs remained free of recurrence for a longer time compared to those not on NSAIDs (**50.6 versus 78.5 months)**. This trend was non-significant statistically, but of relevance in the clinical setting.

The majority of NSAID users and nonusers were ER positive and PR positive and had invasive ductal carcinoma breast cancer. Most NSAID users and nonusers were Hispanic and of White ethnicity.

More NSAID users were on aromatase inhibitors than NSAID nonusers ($p<0.001$). Both NSAID users and non-users were more likely to have lumpectomies, followed by mastectomies and bilateral mastectomies.

Aspirin was the most common type of anti-inflammatory drug among NSAID users. More NSAID nonusers had diabetes than NSAID users. Metformin was the main drug used to treat diabetes in this population.

Although, in general, most patients did not use omega-3 fatty acids or statin, more NSAID users consumed omega-3 fatty acids and took statin drugs than nonusers.

Before constructing logistic regression models, the distribution of patients by BMI categories by NSAID use and recurrence rates was analyzed. **Table 2** shows the numbers and frequencies of how recurrence rates were affected by BMI and use of NSAID. Since the majority of the patients were overweight and obese rather than normal weight (385 compared to 75, respectively), the recurrence rates shown in Table 1 seem to have occurred mainly among overweight and obese breast cancer women. Overweight and obese NSAID users had approximately half recurrence rates as nonusers (6.7% versus 12.9%).

Table 1 Descriptive characteristics of breast cancer cases (N=440)

Characteristics	**NSAID nonusers (n=281)**	**NSAID users (n=159)**	**p-value**
Age at diagnosis, years (mean ± SD)	55.5 ± 10.3	60.7 ± 10.7	< 0.001
BMI, kg/m^2 (mean ± SD)	30.7 ± 6.21	31.9 ± 6.70	0.072
Time to recurrence, months (median)[a]	50.6	78.5	0.464
	|---------- N(%) ----------|		**χ^2 , p-value for trend**
Recurrence			0.050
Yes	34 (12.1)	10 (6.3)	
No	247 (87.9)	149 (93.7)	
BMI categories			0.132
Normal	50 (17.8)	25 (15.7)	
Overweight	94 (33.4)	41 (25.8)	
Obese	137 (48.8)	93 (58.5)	
Menopausal status			<0.001
Premenopausal	110 (39.2)	32 (20.1)	
Postmenopausal	171 (60.8)	127 (79.9)	
Ethnicity/Race			0.135
Hispanic	134 (47.7)	74 (46.6)	
White	111 (39.5)	57 (35.8)	
African-American	5 (1.8)	10 (6.3)	
Other	4 (1.4)	1 (0.7)	
Missing/Unavailable	27 (9.6)	17 (10.7)	
Tumor stage			0.389
I	101 (35.9)	60 (37.8)	
II	107 (38.1)	64 (40.2)	
III	56 (19.9)	23 (14.5)	
Missing/Unavailable	17 (6.1)	12 (7.5)	
Hormone receptor status			0.255
ER+/PR+	246 (87.6)	133 (83.6)	
ER+/PR-	35 (12.4)	26 (16.4)	

Table 1 Descriptive characteristics of breast cancer cases (N=440) (continued)

Characteristics	**Non-NSAID users (n=281)**	**NSAID users (n=159)**	**χ^2 , p-value for trend**
Histological type			0.156
Ductal	218 (77.6)	121 (76.1)	
Lobular	33 (11.7)	21 (13.2)	
Mucinous	1 (0.4)	2 (1.2)	
Missing/Unavailable	29 (10.3)	15 (9.5)	
Type of surgery			0.405
Lumpectomy	128 (45.5)	72 (45.3)	
Mastectomy	122 (43.4)	69 (43.4)	
Bilateral mastectomies	26 (9.3)	12 (7.6)	
Other excision[b]	5 (1.8)	6 (3.8)	
Adjuvant hormonal therapy			<0.001
Aromatase Inhibitors	171 (60.9)	125 (78.6)	
Tamoxifen	110 (39.1)	34 (21.4)	
Type of NSAID[c]			
Aspirin	-	129 (81.1)	
Other drug	-	30 (18.8)	
Diabetes status			<0.001
Diabetic	56 (19.9)	57 (35.9)	
Not diabetic	225 (80.1)	102 (64.1)	
Diabetes Drug[d]			0.036
Metformin	37 (66.1)	33 (57.9)	
Other	19 (33.9)	24 (42.1)	
Omega-3 fatty acids use			<0.001
Yes	32 (11.4)	40 (25.1)	
No	249 (88.6)	119 (74.9)	
Statin use			<0.001
Yes	65 (23.1)	67 (41.1)	
No	216 (76.9)	92 (57.9)	

Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; SD, standard deviation; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor.

[a] Wilcox non-parametric test used to analyze time to recurrence analysis in 44 patients who had a recurrence

[b] Other excision includes segmentectomy and quadrantectomy

[c] Calculations pertinent to the 159 patients classified as NSAID users

[d] Numbers add up to a total of 113 diabetic cases

Table 2 Count of recurrence of normal weight versus overweight and obese NSAID users and nonusers (N=440)

Characteristics	**NSAID nonusers (n=281)**	**NSAID users (n=159)**
	|---------- N(%) ----------|	
Normal Weight (n=75)		
Recurrence	4 (8)	1 (4)
No Recurrence	46 (92)	24 (96)
Overweight and Obese (n=365)		
Recurrence	30 (12.9)	9 (6.7)
No Recurrence	201 (87.1)	125 (93.3)

Analysis for prediction of recurrence

In order to examine whether NSAID use predicted the dependent variable recurrence, five logistic regression models were created. The first model contained NSAID use as the only predictor. In the second model, NSAID use and three BMI categories (normal, overweight, obese) were inserted. The third model included NSAID use, BMI categories and type of hormonal therapy (**Table 2**). A fourth model was created with the predictors from model 3 and the independent variables diabetes status, omega-3 fatty acids use and statin use were added because they were statistically significant different among NSAID users and nonusers, and these three factors have been thought to affect breast cancer risk and outcome. In the fifth model, the variables included were NSAID use, two BMI categories (normal versus overweight and obese) and endocrine therapy. Models using BMI as continuous variable were created, but data is not shown because the numerical variable was non-significant.

As seen in **Table 2**, in model 1, NSAID users were less likely to have breast cancer recurrence than nonusers (OR, 0.48; p-value=0.05). However, this difference was not significant after adjustment for age at diagnosis.

The difference in use of NSAID was borderline significant when BMI categories were inserted in the model. However, NSAID use was non-significant in model 2 after age at diagnosis adjustment. When controlling for NSAID use and age at diagnosis, patients classified as overweight were 3 times as likely than normal weight patients to recur (p=0.04). When compared to obese patients, overweight females were 2 times as likely to have breast cancer recurrence (OR, 2.08; 95% CI, 1.06-4.14; data not shown), when NSAID use was held constant.

In model 3, NSAID users and nonusers did not differ in rates of recurrence when holding BMI categories and type of hormonal therapy constant. After adjustment for age, overweight patients were 3.36 times as likely to have breast cancer recurrence than normal weight patients, when controlling for NSAID use and endocrine therapy. Obese patients were 1.68 times are likely to recur compared to normal weight patients, but this trend was non-significant. Patients who used aromatase inhibitors were about half as likely (OR, 0.47; 95% CI, 0.23-0.94; p=0.03) to recur than those on tamoxifen, when holding NSAID use, BMI categories and age at diagnosis constant.

In model 4, NSAID users had a trend of being less likely to have a recurrence, but the difference was still non-statistically significant when adjusting for confounders and when controlling for the remainder independent variables. Overweight patients were 3.30 times as likely than normal patients to have a recurrence (95% CI, 1.10-12.3; p=0.04), when controlling for NSAID use and the other predictors and adjusting for age and tumor stage. Women using aromatase inhibitors were less likely to have a recurrence compared to those using tamoxifen (OR, 0.40; 95% CI, 0.18-0.83, p=0.01), when controlling for the other predictors and confounders. Diabetic patients were 2.57 times as likely to have a recurrence than non-diabetics (OR, 2.57; 95% CI, 1.15-5.76, p=0.02), when holding the other variables constant. Finally, the use of omega-3 fatty acids and statin drugs did not statistically significant predict recurrence when controlling for the other independent variables.

In model 5, overweight and obese patients were 2.63 times as likely to have a recurrence than normal patients when controlling for the remainder predictors and confounders, but this trend was not statistically significant (95% CI, 0.95-9.44; p=0.09). Females using aromatase inhibitor use were less likely to develop a recurrence compared

to females using tamoxifen (OR, 0.40; 95% CI, 0.20-0.81, p=0.01), when holding NSAID use, BMI category, age at diagnosis and tumor stage constant.

Table 3 Logistic regression model to predict breast cancer recurrence

	Unadjusted			Adjusted for age at diagnosis			Adjusted for age[a] and tumor stage		
Predictors	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Model 1									
NSAID use									
Users	0.48	0.22-0.98	0.05	0.55	0.24-1.13	0.12	0.57	0.24-1.26	0.18
Nonusers	reference			reference			reference		
Model 2									
NSAID use									
Users	0.51	0.23-1.03	0.07	0.58	0.25-1.20	0.16	0.61	0.25-1.36	0.24
Nonusers	reference			reference			reference		
BMI category									
Normal	reference			reference			reference		
Overweight	2.55	0.98-7.93	0.07	3.09	1.11-10.9	0.04	3.39	1.06-15.0	0.06
Obese	1.24	0.47-3.88	0.67	1.48	0.52-5.27	0.49	1.64	0.51-7.32	0.45
Model 3									
NSAID use									
Users	0.58	0.26-1.20	0.16	0.63	0.27-1.32	0.23	0.67	0.27-1.51	0.35
Nonusers	reference			reference			reference		
BMI category									
Normal	reference			reference			reference		
Overweight	2.71	1.03-8.49	0.05	3.36	1.20-12.02	0.03	3.86	1.19-17.46	0.04
Obese	1.35	0.51-4.28	0.56	1.68	0.59-6.04	0.37	2.00	0.61-9.14	0.29
Hormonal therapy									
Aromatase inhibitor	0.48	0.25-0.92	0.02	0.47	0.23-0.94	0.03	0.33	0.15-0.71	0.004
Tamoxifen	reference			reference			reference		
Model 4									
NSAID use									
Users	0.53	0.23-1.14	0.12	0.56	0.24-1.23	0.17	0.55	0.23-1.23	0.16
Nonusers	reference			reference			reference		

Table 3 (continued)

BMI category									
Normal	reference			reference			reference		
Overweight	2.47	0.93-7.82	0.08	2.98	1.04-10.7	0.05	3.30	1.10-12.3	0.04
Obese	0.97	0.35-3.16	0.96	1.14	0.38-4.24	0.82	1.38	0.44-5.32	0.59
Hormonal therapy									
Aromatase inhibitor	0.45	0.23-0.88	0.02	0.45	0.22-0.92	0.03	0.40	0.18-0.83	0.01
Tamoxifen	reference			reference			reference		
Diabetic status									
Diabetic	2.78	1.30-5.94	0.007	2.97	1.36-6.54	0.006	2.57	1.15-5.76	0.02
Non-diabetic	reference			reference			reference		
Omega-3 Use									
Yes	0.69	0.19-1.89	0.51	0.74	0.21-2.04	0.60	0.76	0.12-2.15	0.63
No	reference			reference			reference		
Statin Use									
Yes	0.91	0.41-1.91	0.82	0.98	0.44-2.09	0.96	1.03	0.44-2.29	0.93
No	reference			reference			reference		
Model 5									
NSAID use									
Users	0.55	0.24-1.12	0.21	0.59	0.26-1.22	0.17	0.57	0.25-1.23	0.17
Nonusers	reference			reference			reference		
BMI category									
Normal	reference			reference			reference		
Overweight + Obese	1.86	0.76-5.62	0.11	2.31	0.87-8.01	0.12	2.63	0.95-9.44	0.09
Hormonal therapy									
Aromatase inhibitor	0.47	0.24-0.90	0.02	0.45	0.22-0.90	0.02	0.40	0.20-0.81	0.01
Tamoxifen	reference			reference			reference		

Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio; CI, confidence interval; BMI, body mass index

[a] Adjustment for age at diagnosis

DISCUSSION

In this exploratory study, NSAID patients were more likely to be older, postmenopausal and slightly obese compared to nonusers. This trend was also observed in the LACE cohort (47). NSAID users were approximately five years older than nonusers, but it is not anticipated that this had a significant impact on clinical outcome because disease prognosis is worse among younger women (57, 58). In contrary to younger age, higher age is linked with ER-positive breast tumors (59, 60). Because age at diagnosis could have been a confounding variable between NSAID use and recurrence, given that older women might use more analgesics to relieve joint pains, the created logistic regression models were adjusted for age.

Results of ethnicity/race, tumor stage, hormone receptor status did not differ among NSAID users and nonusers, which contribute to reduced comparison bias.

In accordance with other three studies, NSAID users were less likely to develop breast cancer recurrence compared to NSAID nonusers. Holmes et al. (48) showed that current aspirin use (6 to 7 days per week) was associated with reduced risk of distant recurrence. Kwan and colleagues reported that ibuprofen alone decreased recurrence risk (RR, 0.96; 95% CI, 0.32-0.98), and that non-aspirin NSAID combined with ibuprofen also lowered risk of recurrence (RR, 0.56; 95% CI, 0.33-0.95) (47). In contrast, Li and colleagues did not found this association (50). However, only pre-diagnostic use of NSAIDs was analyzed, so it is unknown how use of NSAIDs after diagnosis of breast cancer may have impacted their disease prognosis.

Breast cancer recurrences were reduced by half in NSAID users compared to nonusers in the chi-square analysis involving all patients. When the logistic regression

models were adjusted for age and tumor stage, NSAID use was associated with lower breast cancer recurrence, but this difference was no longer statistically significant.

Approximately 25% of NSAID users were also taking omega-3 fatty acids compared to 11% of nonusers. This study lacked power to investigate if NSAID use in combination with omega-3 fatty acids added an extra benefit in reducing recurrence rates. Omega-3 fatty acids compete with arachidonic acid as substrates for enzymes that synthesize PGE_2 and other pro-inflammatory agents (61). Therefore, it is likely that the concomitant use of NSAIDs and omega-3 fatty acids, at approved levels, might improve patient outcome by decreasing inflammation. The impact of statin drugs on breast recurrence is inconclusive (62). These drugs block the enzyme HMG-CoA reductase involved in biosynthesis of cholesterol, which decrease vascular inflammation (63), and could potentially work with NSAIDs to impact breast cancer prognosis.

To date, no previous research has been conducted analyzing the time to disease progression in NSAID users and nonusers following invasive breast cancer diagnosis treated with endocrine therapy. Patients classified as NSAIDs nonusers had quicker time to recurrence at median time of 50.6 months compared to those on NSAIDs, who recurred at a median time of 78.5 months. The trend followed the direction expected, but it did not reach statistical significance. This is due to the small number of people who recurred (only 44 total had recurrence). But these results are encouraging for the clinical setting because patients using COX-2 inhibitor drugs remained disease-free for more than two years compared to those not on these drugs.

This thesis work was more conservative in regards to NSAID type and frequency criteria. Patients who used acetaminophen and other analgesics that marginally block COX-2 pathway were categorized as NSAID nonusers different from other studies (47, 50). Further, only daily use of COX-2 inhibitors fit the study definition of NSAID users,

while others have asked if patients used the drugs at least three days per week (47). Another factor that makes comparisons across studies more challenging is the lack of a standard definition of recurrence, especially because recurrence is easily misclassified (48).

Given our population sample was mainly composed of overweight and obese women, recurrence rates predominantly occurred in these patients (data not shown). The small number of normal weight patients limited our comparison analysis. Across research studies, there is no consistent comparison of breast cancer risk, recurrence or survival across BMI categories. Findings are based on comparisons of BMI < 30 kg/m^2 versus ≥ 30 kg/m^2 (12, 32), BMI < 25 kg/m^2 versus ≥ 25 kg/m^2 (50), BMI < 25 kg/m^2 versus ≥ 30 kg/m^2 (64) or using tertiles or quartiles (7).

Furthermore, it is unclear how overweight breast cancer patient with a BMI of 29.50 kg/m^2 and obese patient of BMI of 30.2 kg/m^2 differ in their inflammatory status. Our findings showed that overweight women were more likely to recur compared to normal weight women. Obese women were also more prone to develop recurrence than normal weight, but this trend did not reach statistical significance. In fact, it has been suggested that crown-like structures in the breast of women may be a better biomarker of inflammation than BMI categories (42).

Because of the small size, further comparisons by obesity levels (I, II and III) were not possible. Kwan et al. reported that morbidly obese women (BMI ≥ 40 kg/m^2) were at higher risk of all causes of death (HR, 1.81; 95% CI, 1.42-2.32) and breast cancer mortality (HR, 1.40; 95% CI, 1.00-1.96) (56). Sestak and colleagues found that women with BMI above 35 kg/m^2 had a righter rate of breast cancer recurrence than those with BMI < 23 kg/m^2 (HR 1.39; p=0.03) (7). Subsequent analyses in a larger cohort are needed to compare recurrence across different obesity levels.

These results also confirmed that the use of aromatase inhibitors was associated with improved disease outcome in patients overall, as previously reported by Sestak (7) and Howell (65).

Strengths of this study included height and weight measurements obtained at the clinics rather than self-reported; confirmation of use of anti-inflammatory drugs by examination of several progress notes; conservative definition of recurrence (excluding secondary primaries); inclusion of cases whose type of first line of endocrine therapy were available.

Some of the limitations are the retrospective design; low power that resulted in wide confidence intervals; and reliance on BMI as measure of adiposity. BMI and body fat levels are correlated, but a biological marker of inflammation might help treatment of breast cancer patients. Due to reduced power, this study could not compare recurrence rates among normal weight versus overweight and obese women by NSAID use. Additionally, the majority of the patients were Hispanic, not primarily Whites/Caucasians (66-68). Therefore, the findings should be interpreted with caution and not generalizable to all breast cancer cases in the United States. The results of this exploratory study is in accordance with other reports that showed that breast cancer patients who use NSAIDs had better disease prognosis.

Chapter 5: Conclusions

In this specific population, mainly composed of overweight and obese breast cancer patients, recurrence rates were reduced to half among NSAID users. Equally as important, the use of COX-2 inhibitors delayed time to disease progression in women regardless of their BMI. Because of the low number of normal weight patients, further analysis are needed to investigate the benefit of NSAIDs among obese women compared to non-obese. Despite limited power, the evidence is suggestive of improved disease outcome in breast cancer patients.

The majority of patients were Hispanic, followed by Whites. Thus, this study is not representative of the United States population. Further, few studies have been conducted with African-Americans, who mainly developed estrogen independent diseases. Research with African-American women who use NSAIDs may also increase knowledge on how these drugs impact disease prognosis.

Future directions include a prospective study among women who use both NSAIDs and omega-3 fatty acids. These polyunsaturated fatty acids decrease inflammation by reducing synthesis of PGE_2 (69), so it is important to investigate if patients using both drugs, at approved dosage, would have improved disease outcome.

This was an exploratory study to analyze whether the use of anti-inflammatory drugs affected breast cancer prognosis in patients treated with endocrine therapies. Further research with a larger population dataset is needed to confirm these results.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. NCHS Data Brief 2012(82):1-8.
2. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst 2003;95(16):1218-26.
3. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348(17):1625-38.
4. Loi S, Milne RL, Friedlander ML, McCredie MR, Giles GG, Hopper JL, et al. Obesity and outcomes in premenopausal and postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14(7):1686-91.
5. Sinicrope FA, Dannenberg AJ. Obesity and breast cancer prognosis: weight of the evidence. J Clin Oncol 2011;29(1):4-7.
6. Sparano JA, Wang M, Zhao F, Stearns V, Martino S, Ligibel JA, et al. Obesity at diagnosis is associated with inferior outcomes in hormone receptor-positive operable breast cancer. Cancer 2012;118(23):5937-46.
7. Sestak I, Distler W, Forbes JF, Dowsett M, Howell A, Cuzick J. Effect of body mass index on recurrences in tamoxifen and anastrozole treated women: an exploratory analysis from the ATAC trial. J Clin Oncol 2010;28(21):3411-5.
8. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994;5(2):138-46.
9. Takkouche B, Regueira-Mendez C, Etminan M. Breast cancer and use of nonsteroidal anti-inflammatory drugs: a meta-analysis. J Natl Cancer Inst 2008;100(20):1439-47.
10. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. WMJ 1998;97(9):20-1, 24-5, 27-37.
11. Morimoto LM, White E, Chen Z, Chlebowski RT, Hays J, Kuller L, et al. Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control 2002;13(8):741-51.
12. Majed B, Moreau T, Senouci K, Salmon RJ, Fourquet A, Asselain B. Is obesity an independent prognosis factor in woman breast cancer? Breast Cancer Res Treat 2008;111(2):329-42.
13. van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152(6):514-27.
14. Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. Breast Cancer Res Treat 2010;123(3):627-35.

15. Daling JR, Malone KE, Doody DR, Johnson LG, Gralow JR, Porter PL. Relation of body mass index to tumor markers and survival among young women with invasive ductal breast carcinoma. Cancer 2001;92(4):720-9.
16. Whiteman MK, Hillis SD, Curtis KM, McDonald JA, Wingo PA, Marchbanks PA. Body mass and mortality after breast cancer diagnosis. Cancer Epidemiol Biomarkers Prev 2005;14(8):2009-14.
17. Petrelli JM, Calle EE, Rodriguez C, Thun MJ. Body mass index, height, and postmenopausal breast cancer mortality in a prospective cohort of US women. Cancer Causes Control 2002;13(4):325-32.
18. Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D. Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ 2007;335(7630):1134.
19. Senie RT, Rosen PP, Rhodes P, Lesser ML, Kinne DW. Obesity at diagnosis of breast carcinoma influences duration of disease-free survival. Annals of Internal Medicine 1992;116(1):26-32.
20. Maccio A, Madeddu C, Mantovani G. Adipose tissue as target organ in the treatment of hormone-dependent breast cancer: new therapeutic perspectives. Obes Rev 2009;10(6):660-70.
21. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. Breast Cancer Res Treat 2002;76(1):27-36.
22. Baumgarten SC, Frasor J. Minireview: Inflammation: an instigator of more aggressive estrogen receptor (ER) positive breast cancers. Mol Endocrinol 2012;26(3):360-71.
23. Giuffrida D, Lupo L, La Porta GA, La Rosa GL, Padova G, Foti E, et al. Relation between steroid receptor status and body weight in breast cancer patients. Eur J Cancer 1992;28(1):112-5.
24. Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst 2004;96(3):218-28.
25. Bulun SE, Mahendroo MS, Simpson ER. Aromatase gene expression in adipose tissue: relationship to breast cancer. J Steroid Biochem Mol Biol 1994;49(4-6):319-26.
26. Obiorah I, Jordan VC. Progress in endocrine approaches to the treatment and prevention of breast cancer. Maturitas 2011;70(4):315-21.
27. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med 2003;348(24):2431-42.
28. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56(2):106-30.
29. Jordan VC, O'Malley BW. Selective estrogen-receptor modulators and antihormonal resistance in breast cancer. J Clin Oncol 2007;25(36):5815-24.
30. Nabholtz JM, Buzdar A, Pollak M, Harwin W, Burton G, Mangalik A, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in

postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. J Clin Oncol 2000;18(22):3758-67.
31. Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, et al. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 2011;364(25):2381-91.
32. Wolters R, Schwentner L, Regierer A, Wischnewsky M, Kreienberg R, Wockel A. Endocrine therapy in obese patients with primary breast cancer: another piece of evidence in an unfinished puzzle. Breast Cancer Res Treat 2012;131(3):925-31.
33. Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. Endocr Relat Cancer 2006;13(2):279-92.
34. Siiteri PK. Adipose tissue as a source of hormones. Am J Clin Nutr 1987;45(1 Suppl):277-82.
35. Morris PG, Hudis CA, Giri D, Morrow M, Falcone DJ, Zhou XK, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. Cancer Prev Res (Phila) 2011;4(7):1021-9.
36. Denkert C, Winzer KJ, Muller BM, Weichert W, Pest S, Kobel M, et al. Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer 2003;97(12):2978-87.
37. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2000;89(12):2637-45.
38. Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 2002;62(3):632-5.
39. Nassar A, Radhakrishnan A, Cabrero IA, Cotsonis G, Cohen C. COX-2 expression in invasive breast cancer: correlation with prognostic parameters and outcome. Appl Immunohistochem Mol Morphol 2007;15(3):255-9.
40. Hwang D, Scollard D, Byrne J, Levine E. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst 1998;90(6):455-60.
41. DuBois RN. Aspirin and breast cancer prevention: the estrogen connection. JAMA 2004;291(20):2488-9.
42. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. Cancer Discov 2012;2(4):356-65.
43. Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. Cancer Prev Res (Phila) 2011;4(3):329-46.
44. Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet 2007;369(9573):1603-13.
45. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet 2010;376(9754):1741-50.

46. Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC, et al. Aspirin and the risk of colorectal cancer in women. N Engl J Med 1995;333(10):609-14.
47. Kwan ML, Habel LA, Slattery ML, Caan B. NSAIDs and breast cancer recurrence in a prospective cohort study. Cancer Causes Control 2007;18(6):613-20.
48. Holmes MD, Chen WY, Li L, Hertzmark E, Spiegelman D, Hankinson SE. Aspirin intake and survival after breast cancer. J Clin Oncol 2010;28(9):1467-72.
49. Blair CK, Sweeney C, Anderson KE, Folsom AR. NSAID use and survival after breast cancer diagnosis in post-menopausal women. Breast Cancer Res Treat 2007;101(2):191-7.
50. Li Y, Brasky TM, Nie J, Ambrosone CB, McCann SE, Shields PG, et al. Use of nonsteroidal anti-inflammatory drugs and survival following breast cancer diagnosis. Cancer Epidemiol Biomarkers Prev 2012;21(1):239-42.
51. Fisher B, Jeong JH, Bryant J, Anderson S, Dignam J, Fisher ER, et al. Treatment of lymph-node-negative, oestrogen-receptor-positive breast cancer: long-term findings from National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. Lancet 2004;364(9437):858-68.
52. Huang WY, Newman B, Millikan RC, Schell MJ, Hulka BS, Moorman PG. Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status. Am J Epidemiol 2000;151(7):703-14.
53. Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast 2011;20(1):66-70.
54. Moon HG, Han W, Noh DY. Underweight and breast cancer recurrence and death: a report from the Korean Breast Cancer Society. J Clin Oncol 2009;27(35):5899-905.
55. McTiernan A, Irwin M, Vongruenigen V. Weight, physical activity, diet, and prognosis in breast and gynecologic cancers. J Clin Oncol 2010;28(26):4074-80.
56. Kwan ML, Chen WY, Kroenke CH, Weltzien EK, Beasley JM, Nechuta SJ, et al. Pre-diagnosis body mass index and survival after breast cancer in the After Breast Cancer Pooling Project. Breast Cancer Res Treat 2012;132(2):729-39.
57. Anders CK, Hsu DS, Broadwater G, Acharya CR, Foekens JA, Zhang Y, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol 2008;26(20):3324-30.
58. Gnerlich JL, Deshpande AD, Jeffe DB, Sweet A, White N, Margenthaler JA. Elevated breast cancer mortality in women younger than age 40 years compared with older women is attributed to poorer survival in early-stage disease. J Am Coll Surg 2009;208(3):341-7.
59. van de Water W, Markopoulos C, van de Velde CJ, Seynaeve C, Hasenburg A, Rea D, et al. Association between age at diagnosis and disease-specific mortality among postmenopausal women with hormone receptor-positive breast cancer. JAMA 2012;307(6):590-7.
60. Diab SG, Elledge RM, Clark GM. Tumor characteristics and clinical outcome of elderly women with breast cancer. J Natl Cancer Inst 2000;92(7):550-6.

61. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83(6 Suppl):1505S-1519S.
62. Ahern TP, Pedersen L, Tarp M, Cronin-Fenton DP, Garne JP, Silliman RA, et al. Statin prescriptions and breast cancer recurrence risk: a Danish nationwide prospective cohort study. J Natl Cancer Inst 2011;103(19):1461-8.
63. Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. Trends Mol Med 2008;14(1):37-44.
64. de Azambuja E, McCaskill-Stevens W, Francis P, Quinaux E, Crown JP, Vicente M, et al. The effect of body mass index on overall and disease-free survival in node-positive breast cancer patients treated with docetaxel and doxorubicin-containing adjuvant chemotherapy: the experience of the BIG 02-98 trial. Breast Cancer Res Treat 2010;119(1):145-53.
65. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005;365(9453):60-2.
66. Brewster AM, Hortobagyi GN, Broglio KR, Kau SW, Santa-Maria CA, Arun B, et al. Residual risk of breast cancer recurrence 5 years after adjuvant therapy. J Natl Cancer Inst 2008;100(16):1179-83.
67. Natarajan L, Pu M, Parker BA, Thomson CA, Caan BJ, Flatt SW, et al. Time-varying effects of prognostic factors associated with disease-free survival in breast cancer. Am J Epidemiol 2009;169(12):1463-70.
68. Rosenberg J, Chia YL, Plevritis S. The effect of age, race, tumor size, tumor grade, and disease stage on invasive ductal breast cancer survival in the U.S. SEER database. Breast Cancer Res Treat 2005;89(1):47-54.
69. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 2008;233(6):674-88.

Vita

Ilane Ximenes Frota Máximo was born and raised in Fortaleza, Ceará, Brazil. Her parents enrolled her in English classes shortly after she started reading, and English soon became her favorite subject. This was one of the reasons why she wanted to pursue graduate school in the United States. In 2007, she graduated with a Bachelors of Science in Food Engineering with *Magna cum laude* from the Universidade Federal do Ceará, Brazil. She began her graduate school studies in Nutritional Sciences at The University of Texas at Austin in 2009, and completed her degree in 2013.

Permanent email: ixfmaximo@gmail.com

This thesis was typed by Ilane Ximenes Frota Máximo.